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AN IMPROVED METHOD OF DIAGNOSTICATING DIABETES FROM A DROP OF BLOOD.

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In two previous articles (*Centralblatt f. d. med. Wissensch.*, 1894, No. 49, and Philadelphia *Medical News*, February 9, 1895) I have described a method enabling one to diagnosticate diabetes by the blood. The typical and practical feature of this process is that a drop taken from the tip of the finger in the usual manner by means of a needle permits of an unfailing diagnosis. I have, since publishing the article mentioned, continued my studies, and base the conclusions which I am going to give below on about fifty cases. These comprise both genuine diabetes as well as cases of glycosuria. The test mentioned above has been employed only by a few observers that I know of. Some have communicated with me, and the impression I have gathered from their letters is that its execution is a rather tedious and irksome one, even to the patient microscopist. The difficulty of successfully repeating the experiment lies in the complicated process requisite to the preparation of the staining fluid. This difficulty is enhanced by the fact that the two aniline dyes employed for the test—eosin and methylene blue—vary considerably according to their source. The acidity of the former and the alkalinity of the latter are met with in shifting proportions in the different articles furnished by different manufacturers. Again, the process of preparing the blood specimens by

means of prolonged heating has proved objectionable to many who otherwise would resort to this test. This process consists in subjecting the dried blood specimen (cover-glass preparation) to a heat of 125° to 130° C. for two hours. This is time-consuming work requiring proper apparatus. The copper strip or copper plate ordinarily used is unreliable. The danger of overheating the specimens is very considerable. In this event the test will be negative or doubtful. For these reasons I have tried to simplify the method, and now I am able to announce that I have succeeded in devising a quick and practical process by means of which diabetes can be demonstrated almost as readily by a drop of blood obtained from the tip of the finger by the prick of a needle as it can be done by the urine tests ordinarily employed. Not only is my method nearly as quick as the chemic urinary test or polariscopic one, but it is, I believe, more reliable. Another improvement on the original method is that the microscope is rendered superfluous. Naked-eye inspection is sufficient to make the diagnosis.

It is a well-known fact that by means of dieting and by the administration of certain drugs (antipyrine, for instance, calomel, and ammonium carbonate) the sugar can be made to temporarily greatly diminish or entirely disappear from the urine, even in cases of well-established and undoubted diabetes. Fasting is a tolerably certain means of freeing the urine from sugar. But even in such cases, where the urine test is negative chemically and by the polariscope, my method reveals the diabetic, as I have demonstrated in several instances. The importance of such a test from a clinical point of view, as from that of examinations for life insurance, is obvious. I know from my own observation that diabetics are not infrequently accepted by life-insurance companies as good risks. The so-called danger line of specific gravity, 1·030, is delusive. There are diabetics whose urine is, at times at least, of normal specific gravity.

Diabetes is a much commoner disease than is generally assumed by the profession and the laity. There can, furthermore, be no doubt as to the constant increase of diabetic patients, a fact that has been justly attributed to

the increased strain which modern civilized man is subjected to. It is therefore of importance to have a means of diagnosticating incipient, or threatening, or doubtful dia betes. This, I maintain, can be done from a drop of blood, which, unlike urine, is always obtainable.

The component parts of the reagent are eosin and methylene blue. Saturated watery solutions of these dyes are mixed in about equal proportions, so that a neutral point is obtained; a precipitate forms which is insoluble in water but soluble in alcohol. This precipitate is washed and dried on a filter and reduced to a fine powder. To it are added eosin and methylene blue in small quantities. The amount of each varies according to the respective degrees of acidity and alkalinity of the dyes. Owing to the variability and unreliability in this respect (there are at least four different kinds of eosin to my knowledge), the quantities have to be ascertained by practically and experimentally testing specimens of diabetic and non-diabetic blood. I will state that of the samples I had at my disposal, one twenty-fourth of eosin and one sixth of methylene blue was added to the neutral (dried) compound. This yields a powder of a reddish brown color.

The manner of procedure to make the test is as follows: The drop of blood is procured by quickly pricking the fourth finger of the right hand of the person under examination. The drop is spread either in the usual manner, being placed between two cover glasses, which are withdrawn from each other by means of the fingers or the forceps, taking care to obtain a film of equal thickness (which sometimes is hard to do); or the drop is placed near one of the edges of the square cover slip, held with the left hand, while with another cover slip, held with the right at an angle of about forty-five degrees, the drop is gently spread and then allowed to dry. It is now, for the sake of comparison, placed together with a cover-glass slip preparation of non-diabetic blood in a wide-mouthed bottle or glass jar, such as is usually employed for portable galvanic batteries, containing equal parts of alcohol and ether, say about eight or ten grammes of each. This jar is then placed in hot water contained, say, in a tin cup, where the ether-alcohol is allowed to boil for four minutes. The

boiling point is about 60° C. This is done for the purpose of fixing the hæmoglobin in the red blood-corpuscles. The cover slips thus prepared are now transferred to the test fluid prepared by dissolving about 0·025 to 0·05 of the powder in about ten grammes of thirty-three per cent. of alcohol.*

I must, however, call attention to the fact that this solution retains its characteristic staining qualities only for a few hours, and that for every examination a freshly made solution should be employed. In this fluid the cover slips remain for about four minutes. They are then washed in water, after which it will be found that the diabetic or glycosuric blood film presents a sap or sometimes a bluish-green color, whereas the non-diabetic blood preparation looks reddish-violet.

The contrast is very striking, and is as plain as that which exists between diabetic and non-diabetic urine treated after the usual method, say with Nylander's solution.

This specific reaction I have obtained in about fifty cases of diabetic and glycosuric blood. (Herein are included, however, the cases examined with the method previously published.) Usually the blood test was resorted to first and was followed up, viz., verified, by the urine test. Even in such cases where there is only a trace of sugar demonstrable in the urine—for instance, where a slight black precipitate is noticeable with Nylander's solution only after settling on standing for some time—the test is positive. Lastly, there is a class of persons who are not only predisposed to diabetes, but dwell actually on the borderland of this disease, crossing and recrossing the border line. They do not habitually show sugar in the urine. These individuals I call "sugar liners," in analogy with the term "border liners." This "diabetic border liner" or suspect can also be told by my method. Nothing, however, is easier than to pick the diabetic out of any number of persons, well or sick with any other sort of ailment, by means of my method.

* Alcohol of higher grade gives rise to the formation of a great number of small air bubbles formed between the erythrocytes and the plane of the cover glass, and in the protoplasm of the blood-corpuscles, thus interfering with the study of the corpuscles under the microscope.

The peculiar reaction described consists in the selective affinity of a certain substance occurring in red blood-corpuscles of diabetic patients for another substance contained in the color reagent; possibly a specific principle or substance in the diabetic red blood corpuscles forms a new chemical compound with one of the staining principles contained in the eosin-methylene-blue solution. At all events, the red blood-corpuscles of diabetic or glycosuric blood are stained green, whereas those of non-diabetic blood present a purple or madder color. There is no differential staining of the blood plasma, except in so far as the exuded fluid from the red blood corpuscles resulting from the drying process is concerned. The test proves that there must be a substance other than grape sugar on which the characteristic stain depends; for a cover glass smeared with non-diabetic, say healthy blood, when prepared in the usual manner and treated with a solution of grape sugar, does not yield the diabetes reaction on the application of the reagent, nor will the blood of an animal, say a rabbit, that has received two grammes of grape sugar subcutaneously yield the specific reaction within one or two hours after the injection. But the specific reaction is obtained by floating a cover-glass slip with a film of non-diabetic blood on diabetic urine for about ten or fifteen minutes. Here the stain is, too, the characteristic green color. In this case, then, a substance contained in the red blood-corpuscles, perhaps the hæmoglobin, absorbs and incorporates, possibly combines with, the substance that occurs in the urine of diabetes, determining the same chemic reaction as we see in diabetic blood. By treating, then, normal blood with diabetic urine the former can be rendered diabetic so far as the typical color reaction is concerned. Whether this substance is directly toxic or only is instrumental in interfering with oxidation is a question. It is reasonable to infer that the latter is a well-grounded conclusion, being a foreign substance lodged in the oxygen-carriers.

I may state further that I have experimented with all kinds of diabetes and glycosuria, so far as the probable or supposed origin was concerned. The results have been uniform whether the disease was one of the hepatogenous,

hæmatogenous, neurogenous and psychogenous, traumatogenous, pancreatic, or toxic varieties. In all of them the reaction will infallibly be obtained.

I would in particular call attention to artificial or experimental diabetes produced by the administration of phloroglucin to an animal. Of late I have experimented with rats. The phloroglucin rats show the typical reaction, both as to blood and urine. In rats, however, treated with phlorrhizin the specific blood reaction is absent, although the urine shows a sugar reaction with Nylander's solution. The action, therefore, of phloroglucin on the organism is totally different from that of phlorrhizin. (I did not repeat the experiment of floating specimens of non diabetic blood on the urine of phlorrhizin animals.) Whereas the former seems to give rise to all the metabolic abnormities of the blood met with in genuine diabetes, including the white, unstainable, necrotic (?) corpuscles described in my former articles, there is an absence of that peculiar substance in the erythrocytes which gives the characteristic color reaction with the test dyes. This substance is not grape sugar, as remarked before.

Another point of interest is that the lower vertebrates are susceptible of toxic diabetes, as has been demonstrated by other observers. At all events the chicken is. A chicken cock was given phloroglucin, and the diabetes reaction of the erythrocytes could be demonstrated after three days. Unfortunately, I had no frogs at my disposal, and had therefore no opportunity of ascertaining the positive or negative reaction on a still lower rung of the scale of evolution.

Again, it is perhaps not unimportant to state that the blood of the chicken embryo, taken on the fourth and fifth day of incubation, shows the same color reaction of the discoplasm as diabetes blood. I refrain from drawing conclusions and advancing theories.

Among the patients whose blood I have examined, the diagnosis of diabetes (and glycosuria) was generally made by the blood test first, then verified by chemical and polariscopic examinations. Sometimes, when the latter did not reveal any sugar in the urine, owing to the small percentage, the blood would tell the tale, larger and more appre-

ciable quantities being found on successive trials. This would in a measure explain the observation that the degree of gravity of diabetes is often independent of the amount of sugar found in the urine. The sugar may disappear from the urine, but the toxic state of the blood persists.

I have not succeeded yet in diagnosticating diabetes from glycosuria. Possibly this can be done by perfecting the blood-testing methods. I have no doubt that there are other, perhaps simpler, aniline stains than those which I have described. The Ehrlich-Biondy stain, for instance, imparts a light yellow color to diabetic erythrocytes in contradistinction to the brownish-yellow of the non-diabetic blood. With this reagent, too, a naked-eye diagnosis could be made with a little practice, but the contrast is not so striking as that obtained with the reagent described above.

There is one point which I should like to call particular attention to in order to prevent misinterpretation. Non-diabetic blood when spread in an uneven manner, so that ridges or excessively thick layers are formed, may also show a greenish tint. Again, the blood film that is not sufficiently hardened either by the ether-alcohol or by heat may give doubtful results. Lastly, the dichroism of the erythrocytes must be taken into consideration—*i. e.*, the fact that blood spread on glass appears red on reflected and greenish on transmitted light. But if these sources of possible error are avoided the test is absolute.

Chloral or chloralamid glycosuria is not comprised in my experiments, nor is experimental curare diabetes.

The method described has the disadvantage that minute crystals, which, during the staining process, are precipitated on the specimen, mar the latter somewhat for microscopic examinations. It seems that a *status nascendi* for the newly forming compound of eosin and methylene blue is indispensable to the reaction. Solutions kept in ordinary alcohol lose the quality of the differential stain. Hence the injunction to prepare a fresh solution for every test to be made. The older the test solution is the more uniform —*i. e.*, the less differentiated—is the color of blood specimens of diabetic and non-diabetic blood. Both assume a bluish tint. In order to obtain the finer histologic features

of the blood, diabetic or non-diabetic, especially the differential staining of the nuclei and the granulations of the white blood-corpuscles, ten minutes' staining is requisite.

The chief results of my investigations of the blood in diabetes, both clinical and experimental, briefly stated, are :

1. The diagnosis of diabetes from a drop of blood can be made with as great a certainty, perhaps with a greater one, and almost as quickly, as by the urine tests.

2. There is a substance, at present unknown, which occurs in the diabetic red blood-corpuscles, foreign to these bodies in the physiological state, which causes the specific reaction with the eosin-methylene-blue compound described. In no other condition of the blood is this substance (or combination) met with than in diabetes and glycosuria. The only exception I have met with so far is in embryonic chicken blood.

3. It is not the presence or excess of sugar in the blood which causes the clinical symptoms of diabetes, but it is with greater probability the foreign element alluded to, which is probably combined with the hæmoglobin, in conjunction with the white (necrotic) masses resulting from the decay of the corpuscular elements of diabetic blood, that makes the clinical symptoms and is possibly the anatomo-pathological substratum of the disease.

Postscript.—The results of the blood-tests detailed above had been so uniform that I was firmly of the opinion that in every case where sugar could be demonstrated in the urine the characteristic blood-reaction could be obtained. In one case, however, the test has been negative, although it is clearly one of diabetes, showing 6·5 per cent. of sugar. It is the case of a boy, sixteen years old, who, at the age of fourteen, became diabetic in consequence, as he alleged, of an electric shock. I shall describe this case in detail. A cover-glass preparation of normal blood floated on the urine of this patient did not show the characteristic green color. Since the blood-reaction was normal, I take it to be analogous to phlorrhizin or essential kidney diabetes. There are, then, at least two distinct kinds of diabetes that can be diagnosticated by the blood-test—hæmatogenous (broadly stated) and renal diabetes. The recent experiments of an Italian observer with oxygen on diabetics would practically bear out the conclusions arrived at by me—that, with rare exceptions, diabetes is a disease of the red blood-corpuscles. It seems reasonable to infer that an abundant supply of oxygen—for instance, inhalation of oxygen—will benefit

those patients in whom the oxygen-carriers are hampered in their function.

Description of Plates A and B.

The plates represent cover-glass preparations, of A (with madder-colored erythrocytes), normal, B (with green-colored erythrocytes), diabetic blood. The specimens were heated for two hours to 125° to 130° C. (The ether-alcohol process yields the same pictures.) They were stained in the same eosin-methylene-blue solution according to directions given in the preceding article.

a, leucocyte (epsilon cells—Ehrlich); *b*, eosinophilous cells (alpha cells—Ehrlich); *c*, smallest lymphocytes, showing a delicate seam of protoplasm; *d*, medium-sized lymphocytes, with basophilous granulations; *e*, large lymphocytes; *f*, platelet patches. The platelets (smallest blue bodies) in A show small white globules: in B these are larger and of irregular shapes. *g*, red blood-corpuscles. Those marked with letters on Plate A have small white dots in the central portion which I have called stigmata of the erythrocytes (see *Arch. f. mikrosk. Anatomie*, vol. xxxv, p. 441). It will be seen that some of the red blood-corpuscles have distinct dots, colored the same as the protoplasmic bodies of the erythrocytes. I consider them the atrophied or senile nuclei of the red blood-corpuscles. The erythrocytes show varying degrees of tinctorial power in the normal as well as in diabetic blood.